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THE REGENERATION OF NERVE AND MUSCLE IN THE SMALL INTESTINE

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BY
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THE REGENERATION OF NERVE AND MUSCLE IN THE SMALL INTESTINE

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THE solution of more than one problem in physiology awaits a wider understanding of the nerve plexuses in the intestine. This knowledge would be particularly valuable in interpreting certain observations on the heart. The zig-zag experiments of Engelmann, the bridge experiments of Porter and Fredericq, and the observation that contraction spreads in all directions from the point of stimulation have forced those holding the neurogenic conception of the heart beat to assume that automaticity and conduction depend upon nerve plexuses in the heart muscle. Whether this assumption is correct can be told only after the physiology of these tissues is better known.

The present investigation began in an attempt to determine whether Auerbach's plexus would regenerate. It seemed desirable to know if in this particular the plexus differed from the central nervous system. From this prime object the work was necessarily extended to the regeneration of muscle.

Methods.

Cats and dogs were used in these experiments. The general plan was to make transections of the intestine, allow time for regeneration, and then test for the passage of peristalsis across the line of section. That the passage of peristalsis would be sufficient evidence of regeneration was assumed from the work of Bayliss and Starling and of Magnus, who have shown that intestinal movements depend for their conduction on the plexus of Auerbach. It is well known that after recovery

[1] I wish to express my indebtedness especially to Dr. Carlson of the University of Chicago as well as Dr. Erlanger of the University of Wisconsin, who have given advice and help in the work.

from transection food passes along the intestine in an apparently normal fashion, but whether peristaltic waves pass the line of suture or whether the food is ever after simply crowded through this region, has never been reported. To eliminate as far as possible any experimental errors cats were also studied under the X-ray. Histological examinations were made in all cases.

Under ether anæsthesia the abdomen was opened and a loop of the small intestine raised to view. Stitches were laid at once and the intestine then cut across between and beneath the stitches. The intestine was completely transected, and to make sure, the cut was carried a short distance into the mesentery. An end to end anastomosis was then quickly made by drawing the stitches tight. Black silk thread was used. Recognition stitches were placed on each side of the suture, and in these a loop of silver wire was tied in those cases intended for X-ray examination. The work was done aseptically, and in every case reported the recovery was rapid and without any particular incident. The animals were tested for peristalsis in from two to two hundred and forty days.

In testing for peristalsis a tracheotomy was made and the animal kept under light ether anæsthesia. The skin over the abdomen was opened along the middle line and the cut edges tied to an iron ring, thus forming a cavity in which the intestines could be kept under warm saline. This is the excellent technique for studying intestinal movements suggested by Meltzer and Auer.[2] When this cavity had been filled with normal salt solution at body temperature, the abdomen was opened and the transected loop was raised. The loop was laid over a small platform of cork which had been placed deep in the abdomen by means of a steel rod serving as a support. Graphic records were made by attaching one light lever to a point 1 cm. above the line of section and another an equal distance below, in such a way that the writing point of the lever rose when the circular coat of the intestine contracted.

All physiological workers know the difficulty of producing regular peristalsis in the intestines of an animal with the abdomen opened. A state of inhibition at once ensues accompanied usually by a great loss of tone. This is due to a reflex from the injured and exposed portions, the inhibition of some peripheral mechanism, or possibly to a state of

[2] Meltzer and Auer: This journal, 1907, xx, p. 259.

acapnia as recently suggested by Henderson.[3] This pronounced inhibition when the intestine is exposed to air is only slightly lessened by opening under normal salt solution. In our problem we were under the further necessity of having the peristalsis appear in a given loop of the intestine. Occasionally peristaltic waves are seen in animals under our experimental conditions, but rarely in our experience did the waves appear at the point desired. Stimulation by sodium chloride according to the Nothnagel method, by pinching with forceps, or by induced electrical currents all produced local contractions which seldom if ever resulted in travelling waves.

The first experiments were made on cats, and these animals proved particularly refractory in regard to intestinal movements. This finding parallels that of Bayliss and Starling,[4] who report in the majority of experiments in cats an almost complete absence of local reflexes. A number of methods were tried to obviate this difficulty. Henderson's[5] method of opening the abdomen in an atmosphere of carbon dioxide did not prove very satisfactory in our hands, although it was evident that it prevented the usual excessive loss of tone. The use of barium chloride as recommended by MacCallum[6] also gave negative results. Finally the injection of eserine salicylate was resorted to. This drug was used in preference to pilocarpine, since the latter seemed to produce mainly pendular movements and very seldom peristalses that travelled any distance. A one quarter grain tablet of eserine was dissolved in 20 c.c. of normal salt solution and injected in the external jugular vein in doses of 1–2 c.c. as needed.

It is assumed that under the influence of eserine conduction takes place by the same mechanism as in normal movements. This assumption seems justified, since the waves produced by eserine resemble normal waves in all important particulars. An increased rate of conduction is the chief difference. To be quite sure, however, the results were controlled by X-ray examinations.

Details in regard to the X-ray observations and the histological technique will be given later in the paper.

[3] HENDERSON: This journal, 1909, xxiv, p. 66.

[4] BAYLISS and STARLING: Journal of physiology, 1901, xxvi, p. 125.

[5] HENDERSON: *Loc. cit.*

[6] MACCALLUM: This journal, 1904, x, p. 259.

PERISTALTIC WAVES PASS THE TRANSECTION.

The first experiments were made on eight cats. In all these the small intestine was transected and an end to end anastomosis made in the way described above. The preliminary operations were done in November and December, 1908, and the observations on peristalsis were made in May and June.

The first two cats studied may be passed briefly. Cat No. 1 was tested for the passage of peristalsis fifty-three days after transection. The animal had been suffering with some pneumonic complaint and was in such poor condition that it died while being placed in a bath of normal saline. The basin made by sewing the skin to an iron ring was not used in these first two experiments. An attempt was made to stimulate peristalsis by pinching and by applying salt crystals. Contractions were produced, but they remained local. Cat No. 2 was examined one hundred and nine days after the preliminary operation. The animal was mangy, poor, and in bad condition generally. The abdomen was opened under normal saline, and typical responses were secured by mechanical stimulation and with sodium chloride. Inhibition below and contraction above the point of stimulation were evident.

The remaining six cats of this first series were in perfect condition when tested for peristalsis. So uniform were the results and the technique that only two protocols will be given.

Cat No. 6. — December 26. Intestine transected. Recovery rapid and uneventful.

May 12. One hundred and thirty-seven days later tested for peristalsis.

2.30 P. M. Ether anæsthesia. Tracheotomy. Skin of abdomen opened along median line, reflected and sewed to iron ring. Cavity thus made filled with warm saline.

2.50 P. M. Abdomen opened under salt solution and transected loop drawn out. Slight adhesions of omentum along line of suture cleaned away. Intestine is enlarged above line of section, but only slightly so below.

3.10 P. M. 1 c.c. of eserine injected in external jugular.

3.13 P. M. Strong pendular movements.

3.20 P. M. $1\frac{1}{2}$ c.c. eserine injected.

3.21 P. M. Peristaltic waves in various loops.

3.22 P. M. Peristalsis started just below suture line.

3.25 P. M. Peristalsis appeared above, but died away.

3.41 P. M. Antiperistalsis passes up intestine crossing line of section.

3.45 P. M. 1 c.c. eserine, followed by tonic spasm of whole intestine.

4.04 P. M. Peristalsis passed line of section. No delay.

4.05 P. M. Peristaltic rush swept down intestine passing through transected loop.

4.15 P. M. Cat pithed. Canal opened in lower cervical region.

4.20 P. M. Peristalsis passed. No delay. (Tracing shown in Fig. 1.)

4.21 P. M. Peristalsis passed. Short delay.

4.23 P. M. Antiperistalsis passed through loop.

4.50 P. M. Wave reached line of section: short delay, then passed through.

5.20 P. M. Cat killed. Loop with line of section removed and placed in normal salt solution. One peristaltic wave passed through loop after being placed in saline.

Cat No. 2. — Black female. — December 2. Intestine transected 40 cm. below duodenum. Recovery uneventful.

May 13. Abdomen opened aseptically and two silver wires sewed on the line of section a short distance apart. No adhesions. Intestine slightly enlarged at either side of suture line.

May 25. X-ray examination after feeding salmon mixed with bismuth subnitrate. Cat had previously been taught to lie on frame over X-ray machine. Waves were passing over stomach. Duodenum opened after every third or fourth wave. Loop of intestine with wire rings located and separated from the other loops by kneading with the fingers. Wire rings showed as circles with clear centres, and thus marked line of section. Dark mass of food to left of transected area. Latter clear. Mass of food passed to right under wire rings and then on down the intestine for some distance. The transected line was first light, then dark, then light again. That is, peristalsis carried food across the line of section. Rhythmical segmentation was not observed in region of the rings.

June 4. X-ray examination. Stomach and intestines well filled. Emptying movements of stomach clearly seen. Rhythmical segmentation at first in upper part of intestine. Later segmentation observed in transected loop. Movements lasted a minute or more.

June 15. One hundred and ninety-five days after transection, test made for peristalsis.

3.20 P. M. Ether anæsthesia. Tracheotomy. Abdomen opened under saline in usual way. Wire rings found to be in place. Wires with slight adhesions removed.

3.47 P. M. 1 c.c. eserine injected into external jugular.
3.48 P. M. Irregular contractions, lasting several minutes, but followed by orderly peristalses.
4.02 P. M. Wave blocked at line of section.
4.25 P. M. Series of waves, one about every minute. Two blocked. Third passes.
4.32 P. M. Wave blocked.
4.38 P. M. 1 c.c. eserine. Followed by irregular movements.
4.40 P. M. Strong peristalsis passed line of transection.
4.50 P. M. Cat killed.

X-ray examinations were made in only one animal of this series, the one described above. The other experiments gave precisely identical results in each of the six cats. In every case under the influence of eserine, peristaltic waves passed the line of previous section. The intestine in each experiment gave all well-known movements seen after the administration of this drug; that is, irregular movements, peristaltic rush, antiperistalsis, and finally, as the effect of the drug wears off, regular, slowly travelling peristalses. The latter alone were especially noted, since the mechanism of antiperistalsis and peristaltic rush is obscure.

Fig. 1 is a tracing from Cat No. 6, showing the passage of a peristaltic wave. The lever writing the lower line was attached 1 cm. above the line of section toward the duodenum, and the lever writing the upper line, 1 cm. below. Each curve shows a period of inhibition preceding the contraction. That this depression was not due to any movement of the intestine made by the tugging of the advancing wave is shown by its presence in nearly all of the tracings and by its disappearance at the lower lever in cases of block. The tracings show that each lever recorded first a wave of inhibition and then a contraction. The evidence, however, is not conclusive that the inhibitory part of the wave was actually conducted through the lesion. To avoid any disturbance due to the contraction above, the lower lever had to be placed too far away from the line of section to settle this important point. In work now undertaken we hope to clear the matter up by using the enterograph. At any rate, the fact seems beyond question that a peristaltic wave of some kind passed through the transected region.

Ways in which a Peristaltic Wave might pass the Transection.

There are a number of ways in which a peristaltic wave might bridge an injured portion of the intestine. A long reflex through the central nervous system, such as occurs normally in the œsophagus, might be developed in case of necessity. Reflexes through sympathetic ganglia of the abdomen would be another possibility, although the evidence seems against sympathetic ganglia mediating reflexes. Langley and Magnus [7] have also found that degeneration of the mesenteric nerves has no effect on intestinal movements. Muscular regeneration might occur at point of section and a myogenic form of conduction be developed. The passage might be made merely by mechanical tug of the muscle on one side stimulating the muscle on the other. Finally there might be a regeneration of Auerbach's plexus, which is the normal means of conduction.

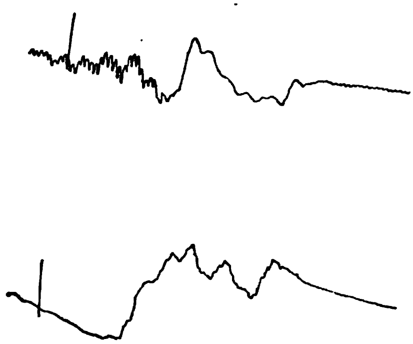

Figure 1. — Showing passage of peristalsis one hundred and thirty-seven days after transection of small intestine. Inhibition preceding contractions is to be noted.

To find which one of these mechanisms the intestine employs after transection now became the real object of the work. The central nervous system was eliminated by studying pithed animals. Four of the six cats had the cord destroyed from the cervical region down. In every animal waves still passed over the suture line. An attempt was made to extirpate the abdominal ganglia, but this proved a difficult procedure, particularly when it was desirable to keep the intestines in saline solution. Fortunately in Cat No. 6 a wave appeared in the loop after it had been removed and placed in salt solution. This definitely eliminated any reflex through extrinsic centres and showed that the mechanism was in the intestine itself.

The X-ray studies described in the protocol were now made to eliminate as far as possible any experimental errors and to decide to what

[7] Langley and Magnus: Journal of physiology, 1905, xxxiii, p. 34.

extent mechanical tension was a factor in the passage of the wave. While it was easily conceived that mechanical stimulation from the impact of a mass of food might start a contraction simulating a true peristalsis, it seemed improbable that this method could account for rhythmical segmentation. Examinations were made repeatedly on Cat No. 2 in the hopes of finding the transected loop in a segmenting condition. As the protocol states, this search was rewarded by a clear picture of segmentation with the line of transection in the centre of the area. This was believed to be strong evidence of nervous regeneration, since the correlation necessary for the complex movements is generally attributed to the nerve plexus. Later work showed, however, that this evidence was not conclusive.

In all the cats of this series there was a slight increase in the diameter of the intestine above the line of section. In three this was rather marked. This hypertrophy is comparable to that found in experiments in which loops of the intestine are reversed in direction. The dilation is probably due to a temporary occlusion of the intestine as the result of the operation and also possibly to a temporary delay in the development of the conducting mechanism, whatever this may be.

The experiments gave some evidence that the power of conduction even at one hundred and thirty-seven to one hundred and ninety-five days was not as perfect as in the uninjured portions of the intestine. Often there was a noticeable delay in the passage of waves through the transected region and at times there was a complete block. These results seem to indicate that although conduction is reëstablished the mechanism is not quite so efficient as formerly. This may mean that another mechanism less capable has taken over the function, or that the conductive tissue has not regenerated completely.

The Interval between Transection and the Return of Conduction

The evidence presented above seems conclusive to us that there is a physiological restoration after transection of the small intestine. The next phase of the problem was to see how soon this regeneration might return. We were led to do this, since it seemed possible to gain some insight as to the mechanism involved in the conduction by learning the time at which waves began to pass through the transected loop.

Cats were again used, and the preliminary operation performed in the usual way. Silver wires were sewed in the stitches so that X-ray studies could be made. Protocols of these experiments will not be given. The technique was the same as in the preceding, and the only point of interest was to find how soon this passage occurred. Cat No. 10 after a quick recovery from the first operation was experimented on eighteen days later. Fig. 2 presents the results. This figure shows the pas-

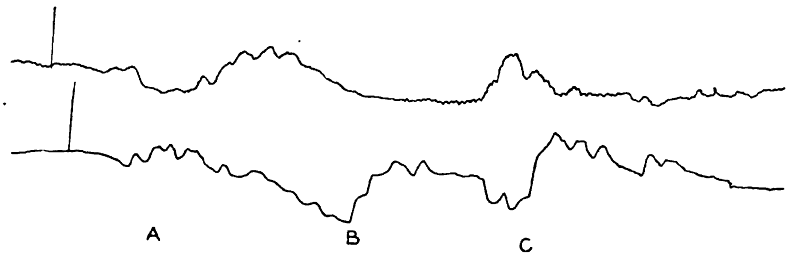

FIGURE 2. — Showing passage of peristaltic waves in a cat eighteen days after transection of the intestine. Lever writing lower line is attached above toward the duodenum.

sage of two peristaltic waves, *A* and *B*, and a block of wave *C*. Wave *A* was preceded by inhibition. This does not show so clearly in the second wave, but it was present in other tracings. There can be no question of nerve regeneration here unless the plexus regenerates in a most remarkably short time. It would seem that the passage must either be due to muscular transmission or mechanical tension.

Cat No. 11 was examined nine days after the first operation. Here too there was passage of the peristaltic wave. Fig. 3 was made in this experiment. Cats Nos. 12 and 13 were tested at four and six days respectively, but the results were negative. Blocking was frequent. Fig. 4 taken from cat No. 13 illustrates this point.

Animals studied under the X-ray confirmed the above results. Cat No. 14 made a poor recovery, and successful observations were not made until the thirtieth day. Dark masses of food were seen passing through the transected loop. Careful watch was kept for rhythmical segmentation, but without result. Cat No. 15 proved a much more successful subject. The animal ate the morning after the first operation and showed absolutely no ill effects. A watch was kept every second day after feeding for the passage of peristaltic waves. On the eighth day peristaltic waves were seen to pass the line of transection, and later in the same day rhythmical segmentation was observed in the loop.

The last result was somewhat unexpected, and we have made no attempt to draw conclusions from it. There may have been no correlation through the line of section. As Dr. Carlson suggests, the chemical and physical consistency of the intestinal contents constitutes the adequate stimulus for segmentation movements. If such is the case, these movements might easily be set up in two adjacent areas separated by complete transection of the nerves and muscular coats. It would be

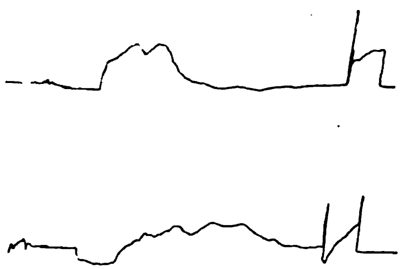

FIGURE 3. — Showing the passage of a peristaltic wave nine days after transection. Upper line from lever attached nearer duodenum.

FIGURE 4. — Showing block of peristalsis in a cat six days after transection of small intestine. Lower lever nearer duodenum.

impossible to tell whether the line of section merely passively divided two such regions or whether there was conduction and correlation.

Transections in a third series of animals, comprising six female dogs, were next studied. No X-ray examinations were made. Otherwise the experiments were carried out in the manner previously described. Dogs Nos. 5 and 6 differed from the others in having only the muscular coats transected.[8] This modification was made by Dr. Carlson to insure a more complete end to end anastomosis than is possible with a complete transection. The cut was made down to the mucosa and entirely around the intestine. The protocols of these experiments are similar to those already given and need not be repeated here. The dog's intestine responds much better to artificial stimuli than that of the cat, but to make the work uniform eserine was again used. Three of the dogs were kept one hundred and eighty days and three two hundred and forty days. Peristalsis was shown to pass the line of transection in each case. Fig. 5 shows the passage in one dog two hundred and forty days after transection. A peristaltic wave may pass the transection by simple mechanical means.

[8] The transections in these animals were made in Chicago by Dr. Carlson and Dr. Werelius, whom I wish to thank for their kindness.

Thus far the work offered practically nothing in solution of our original thesis, the regeneration of Auerbach's plexus. However an advance has been made in discovering that physiological regeneration in the intestine is no proof of anatomical regeneration, so far at least as the nerve plexus is concerned. At first it was thought that the passage of a wave across the suture line would be ample proof of nervous regeneration. Later, when this idea was given up, it seemed equally certain that rhythmical segmentation would be sufficient evidence. It has been shown how this too may take place long before the nervous mechanism could possibly grow anew. All of this, to be sure, by no means disproves a regeneration of Auerbach's plexus. This may take place at the proper time and under the proper conditions. It does show that until recovery is complete the intestine has other mechanisms that enable it to carry on its usual motor function. The real proof for regeneration must be sought by histological methods.

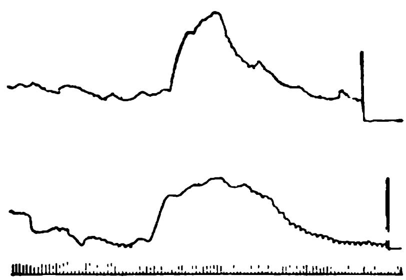

FIGURE 5. — Showing passage of peristalsis in dog two hundred and forty days after transection. Lever writing lower line nearer duodenum.

An attempt was made to find exactly how the early conduction across the injured portion took place. It seemed obvious that it was a case either of mere mechanical tension or of conduction through the muscular tissue. As will be described later, the histological studies showed that the longitudinal coat regenerated very rapidly, and for a time this means of conduction seemed more probable than any other. The matter was finally decided by the following experiment. A cat under ether was arranged in the usual way for studying peristalsis. At a convenient point in the small intestine the muscular coats were transected, the mucosa being left intact beneath. This was done carefully, the muscular coats being divided by cutting a ring around the intestine. The continuity of the intestine was thus preserved by the mucosa and submucosa alone. Five centimetres above the ring a slit was made in the intestine and a similar one an equal distance below. A bolus of cotton smeared with vaseline was inserted in the upper slit. Peristaltic waves were produced by the injection of eserine. The bolus was soon carried

down the intestine by a peristaltic wave, and forced past the narrow ring which was stripped of its muscular coats. With little or no delay a wave appeared on the lower side of the transection and the bolus was crowded down the intestine until it appeared at the lower opening. This result was obtained repeatedly.

In this experiment there could be no question of muscular conduction. Neither does it seem possible that the mucous or submucous coat could be concerned in the conduction of the impulse. Magnus,[9] it will be remembered, found that the mucosa and submucosa gave no movements and took no part in the general motor functions of the intestine. This experiment seems clearly to demonstrate that a peristaltic wave may be conducted across a gap in the intestine by simple mechanical means. The tug of the contracting musculature above the line of transection and the impact of the bolus below is sufficient to set up a contraction, and the wave continues downward.

This is a striking example of mechanical correlation. It illustrates the ability of the body to develop or make use of other mechanisms when any given one fails. We believe this method of conduction is the one used by the intestine after transection until the continuity of the plexus, the normal mechanism, is restored, provided that is ever possible. The rather frequent occurrence of delays at the line of suture may now be explained. Mechanical stimulus is not the natural one for the intestine, and it does not respond as quickly or as accurately to it. The dilation often occurring above the line of transection may be due in part to the slightly diminished efficiency at this point.

Histological Studies.

So far as our original problem is concerned, the most important result obtained is that the passage of the peristaltic wave across the intestine is not a proof of the continuity of the nerve plexus. The decision in regard to Auerbach's plexus must be made on purely histological grounds. For this purpose all of the transected portions in the preceding experiments were studied.

Methylene blue and gold chloride were used to stain the nerve plexuses. Methylene blue proved difficult to handle in the large pieces, and so most of the work was done with gold chloride. The tissues were placed

[9] Magnus: Archiv fur die gesammte Physiologie, 1904, cii, p. 349.

in 1/2 per cent arsenic acid thirty minutes, in gold chloride thirty to forty-five minutes, reduced in 1 per cent arsenic acid over the water bath for ten to fifteen minutes, and preserved in glycerine. To study the muscular coats pieces were fixed in Zenker's fluid, washed, dehydrated, embedded in paraffin, sectioned, and stained with Mallory's muscle stain.

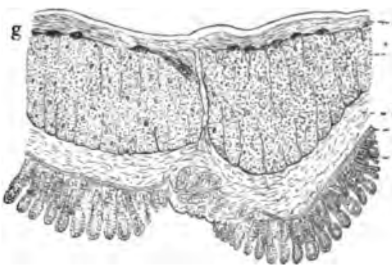

FIGURE 6. — Longi section through line of transection of cat's intestine. Nine days after operation. *a*, longitudinal muscular coat; *b*, circular muscular coat; *c*, submucosa; *d*, muscularis mucosa; *e*, mucosa with villi and glands; *b*, ***mucosa*** disintegrating at point of anastomosis; *g*, ganglionic masses of Auerbach's plexus.

A careful study was made of the regeneration of the different coats of the intestine after transection. At the time considerable importance was attached to the rapidity with which the muscular coats regenerated, since it was believed that they might be the agents of conduction. On finding that the passage was at first due to mechanical factors this part of the work became of secondary importance. It is believed worth while, however, to give the general results.

The work of Mall [10] on the healing of intestinal sutures is well known, and we can confirm him in most details, except in regard to the length of time required for regeneration.' Mall found complete regeneration in dogs only at about sixty days. Recovery in cats is much more rapid. Fig. 6 is a more or less diagrammatic longi section through the line of transection in a nine-day cat. Regeneration is not yet complete, but the process is well on its way. The longitudinal muscular coat at least has regenerated. The circular coat is separated by a heavy band of connective tissue which persists indefinitely. The submucosa has reunited, and new villi and glands are being formed in the injured mucosa. The anastomosis in this case seems to have been well made, and the rapid growth is perhaps due somewhat to this fact.

In every case the regeneration begins with a fibrous union of the serous surfaces. This may take place in a few hours. The longitudinal muscular coat regenerates quicker than any other part except the serosa. Protruding parts of the mucosa are destroyed. The circular muscular coat, strictly speaking, does not regenerate, since it has been

[10] MALL: Johns Hopkins Hospital reports, 1896, i, p. 376.

merely separated by the cut, its fibres running parallel to the plane of the section.

Fig. 7 illustrates a section through a six-day stage. The longitudinal muscular coat has not yet regenerated. At *g* and *h* outgrowing projections may be seen which are to make the connection. In many cases the junction between the separated portions of the longitudinal coat is made by a projection taking the course of *h* in Fig. 7. These sections are of importance in showing the relation the ganglionic masses of Auerbach's plexus bear to each other after the intestine has been transected.

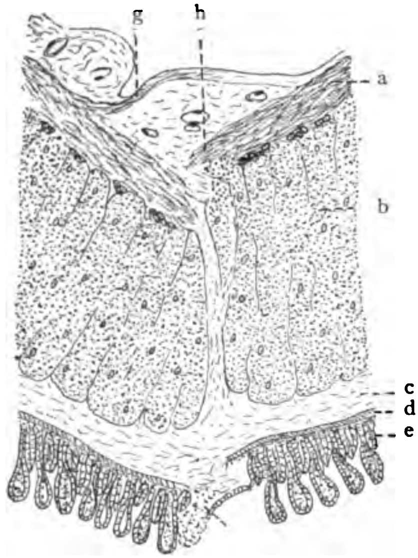

FIGURE 7. — Longi section through line of transection in a cat six days after operation. *g*, regenerating portion of longitudinal muscular coat; *h*, portion of growing muscle carried across by the serosa. Other letters the same as in Fig. 6.

Gold chloride, as every one knows who works with it, is more or less capricious. Animals also vary in the ease with which their tissues are impregnated. Auerbach's plexus in the cat is stained with some difficulty. In our work we were under the further necessity of having the stain appear at a given point. For these reasons the gold chloride stains on the first series of cats were unsatisfactory. A large piece of the intestine with the scar in the centre was stained. The mucosa was either left intact or carefully removed. Adhesions on the serosa were left, since their removal might have damaged the longitudinal coat and the plexus lying immediately beneath. The idea was to stain the plexus on each side of the scar and study to see if any fibres passed across. In the cat intestines the stain did not take uniformly over the entire piece. While we did not demonstrate any regeneration, we feel the results in this first series have little weight, since the stain might have failed exactly where needed.

Auerbach's plexus in the dog stains rather easily, and in the series of dogs from one hundred and eighty to two hundred and forty days

after transection good stains were secured in each case. Six different intestines were studied. The results in the first five of these were negative. The first four were from dogs in which the intestine had been cut entirely across. Dogs Nos. 5 and 6 had only the muscular coats transected. In the first four little could be seen in the scar tissue. Figs. 6 and 7 show how the connective tissue develops between the circular coats and even between the serosa and the longitudinal muscular coat. In the dog this is sometimes very pronounced, especially in those cases in which the transection has been made entirely across the intestine and the anastomosis has been somewhat inaccurate in placing the various layers end to end. Under the influence of the gold this scar tissue darkens, and one can scarcely decide whether or not nerve fibres penetrate it.

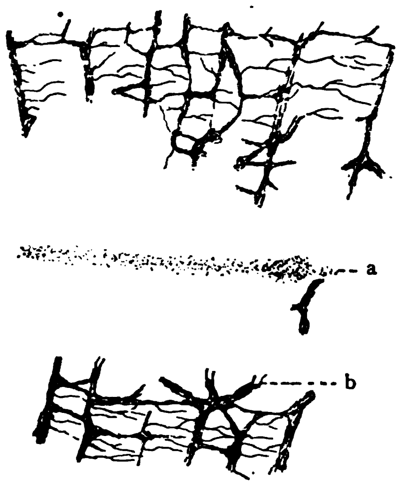

FIGURE 8. — Gold chloride stain of transected area in dog two hundred and forty days after operation. *a*, line of transection; *b*, end of strand of the plexus.

Dogs Nos. 5 and 6 were more favorable for study. In these there was scarcely any thickening at the suture line. The layers had been nicely approximated and scar tissue was at a minimum. Fig. 8 is from the line of transection in Dog No. 5. A slight thickening at the exact line of section is evident. On either side (to be exact, 1½ mm.) are seen the blunt ends of the large strands composing the plexus. On a few of these ends, such as *b*, are faint suggestions of outgrowing fibres, but they cannot be traced any distance. No fibres appear anywhere between or across the scar. The plexus is stained even to fine details in all parts of the preparation, except the blank area on either side of the scar. The nerve cells can be distinguished in the ganglionic masses. It is clear that there has been no regeneration of the plexus.

Dog No. 6 was kept one hundred and eighty days after the circular suture was made. Fig. 9 shows the results of the gold chloride stain. Superficial connective tissue adhesions were practically absent in this specimen. The plexus stained well in all parts of the preparation. The

cut ends of the large plexus strands in this case are not blunt as in the previous one. From the severed ends fibres pass out into the scar area, and at least five of these fibres in the one small region drawn can be seen to pass across and enter the strands on the other side. Many other processes pass out into the scar tissue and are lost to view. High powers of the microscope show that these fibres are not mistaken blood vessels, but real non-medullated nerve fibres. At first it was thought that these fibres might have been some lying deep in the muscular layers and thus escaped section. But this could not be, since on cutting the muscular coats transversely they immediately pull apart and expose the mucosa below. Fibres could not possibly escape both rupture and cutting. Besides the processes in question are well toward the upper surface of the specimen, and the large strands show plainly that the transection was complete. We have here undoubtedly a good example of nerve regeneration.

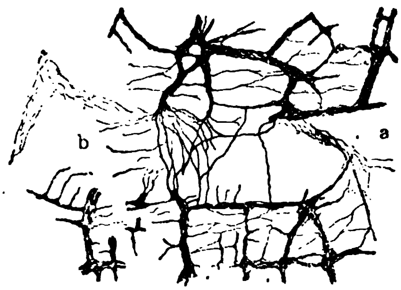

FIGURE 9. — Gold chloride stain of transected area in dog one hundred and eighty days after transection. *a* to *b* marks the line of transection.

A more difficult problem is to determine the origin of the regenerating fibres. Unfortunately very little work seems available on the nervous elements constituting Auerbach's plexus. That extrinsic nerves enter the plexus is well known, but what becomes of them is by no means clear. Dogiel,[11] Cajal,[12] and others believe that the plexus contains two kinds of fibres. These are, first, those coming from ganglionic cells of the plexus and, second, certain "passage fibers" whose origin is obscure. The passage fibres may be merely unusually long axones from ganglionic cells or they may be postganglionic fibres from extrinsic abdominal nerve centres. These passage fibres are of small diameter, less numerous than the others, and characterized by varicosities.

Gold chloride preparations do not allow one to discover the origin of the fibres crossing the scar. The fibres can be easily traced into one of the large plexus strands, and the nerve cells in these strands can be dis-

[11] DOGIEL: Anatomische Anzeiger, 1895, x, p. 517.
[12] CAJAL: Structure du système nerveuse, 1895, p. 140.

tinguished, but it would be hazardous to say just where the processes end. We believe, however, that the fibres are intrinsic and are processes of the ganglionic cells constituting Auerbach's plexus. The chief evidence for this belief lies in the fact that the regenerating fibres are too numerous to be considered passage fibres. Dogiel and Cajal do not state the relative number of the latter, but their figures show only a few in each strand. While only five or six fibres can be traced completely across the scar in our preparation, Fig. 9 shows how large numbers pierce well into the scar tissue. At *a* is a large number of these fibres so crowded that they resemble an entire strand of the plexus. A second reason for believing that the fibres under question are not passage fibres is that their course is direct and they show none of the sinuosities of such axones.

As the matter stands, we believe we have definitely proved the regeneration of certain fibres in Auerbach's plexus. There may still be some question as to the origin of these fibres, but it seems reasonably clear that they are nerve processes from cells in the plexus itself.

Why was there no regeneration in any of the other animals studied? An answer to this question can only be problematical, remembering at the same time that one positive experiment is worth any number of negative ones. There is no doubt that an entire transection of the intestine is unfavorable to regeneration. Scar tissue is extensively produced, and this is doubtless difficult to penetrate. What is even more important is that in this procedure only occasionally are the layers closely approximated in the anastomosis. A condition shown in Fig. 7 is usually produced. The longitudinal coat reunites by a new path (see *b* in Fig. 7), and the cut ends of the plexus are so far removed and separated by the muscular coats that regeneration could hardly be expected. Cutting only the muscular coats allows a close approximation of the ends of the plexus, and conditions are far more favorable. Age may also be a factor. One would expect regeneration to occur more easily in the young than the old. This factor is being further investigated.

The regeneration of Auerbach's plexus suggests that this may occur in other plexuses, and thus opens up a new line of investigation of the heart. Erlanger[13] has produced artificial heart-block in dogs by crushing the bundle of His with the clamp especially devised for that purpose. The dogs were allowed to recover and lived two hundred and

[13] ERLANGER, BLACKMAN, and CULLEN: This journal, 1908, xxi, p. xxviii.

sixty-nine and two hundred and seventy-eight days in a state of chronic heart-block. The same author has recently reported [14] separating a portion of a dog's auricle from the remainder of the musculature by crushing. Two hundred and sixty-eight days after this operation the heart was exposed and the portion isolated by crushing stimulated electrically without the rest of the heart being affected. Judging from our results, one might expect regeneration of plexuses in heart tissue. The operative methods described above should be duplicated and followed by a careful study of the plexuses in the heart musculature. This will be a difficult problem, considering the unsettled state of our knowledge concerning nerves in the heart, but it is being undertaken with some prospect of ultimate success. It may yet be possible to subject the neurogenic and myogenic theories to a crucial test in the vertebrate heart.

Summary.

1. The small intestine was transected in cats and dogs in order that the regeneration of Auerbach's plexus might be tested.

2. Physiological restoration, as determined by the passage of peristalsis across the lesion and by segmentation movements, has been demonstrated from the eighth day.

3. This physiological restoration is not a sufficient test for the regeneration of the nervous mechanism in the intestine.

4. There is no reason for doubting that the continuity of Auerbach's plexus is necessary for the normal intestinal movements, but other methods may be employed when the nervous mechanism is injured. In the cat it has been definitely shown that after transection the peristaltic wave may be conducted by mechanical means. A stimulus is found in the tug on the musculature or on the nervous elements by the contracting ring above the section and in the impact of the bolus.

5. The longitudinal coat of the cat's small intestine may regenerate in from seven to nine days after a circular suture.

6. In one of six dogs a regeneration of Auerbach's plexus was shown one hundred and eighty days after transection of the circular and longitudinal coats of the intestine.

[14] Erlanger: This journal, 1909, xxiv, p. 375.